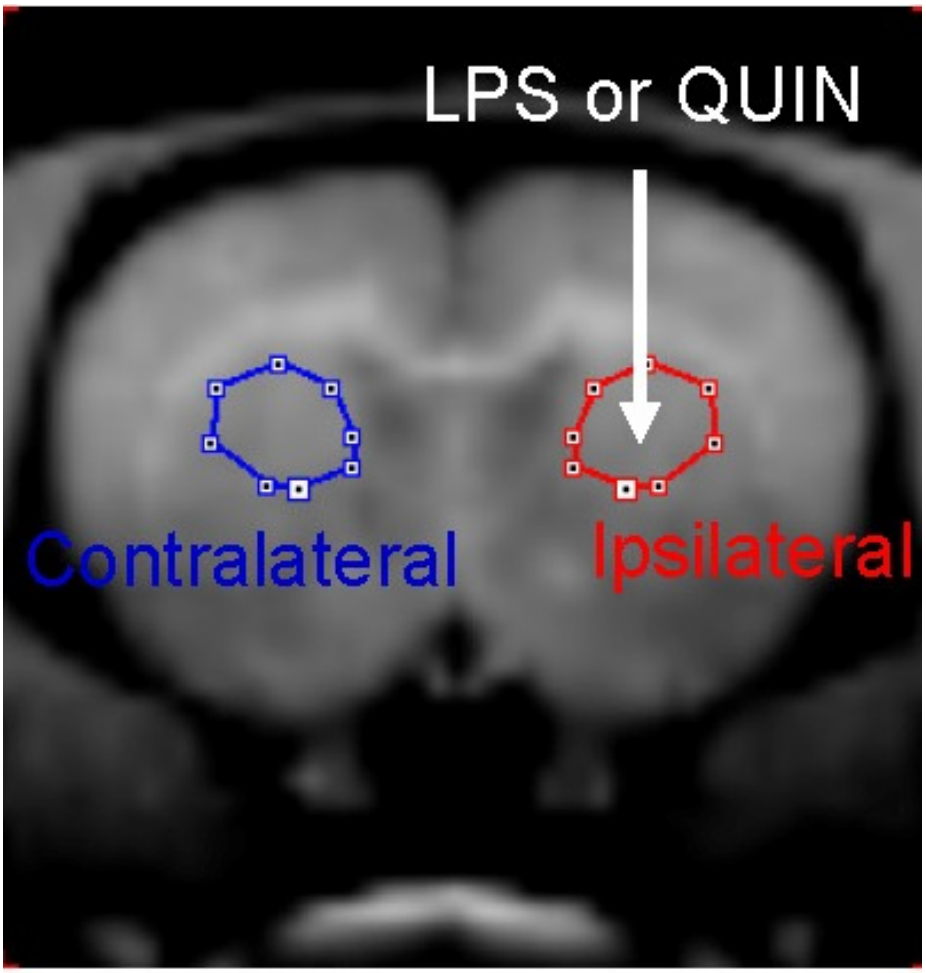
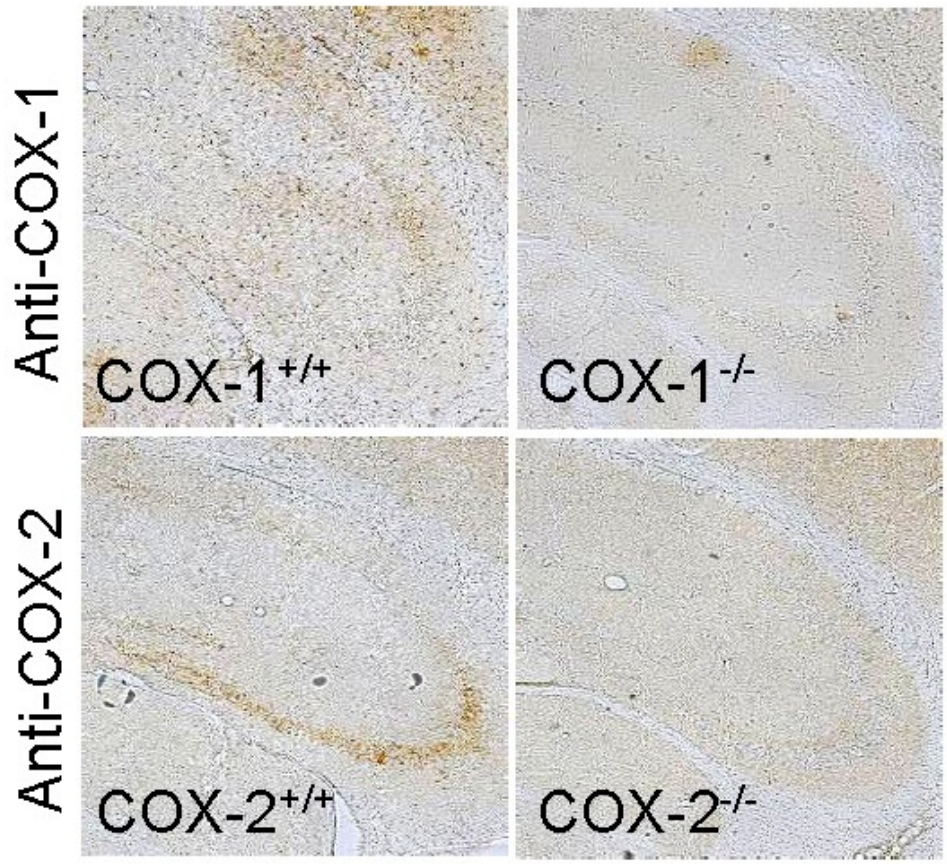
Supplemental Material and Methods

Western blot analysis

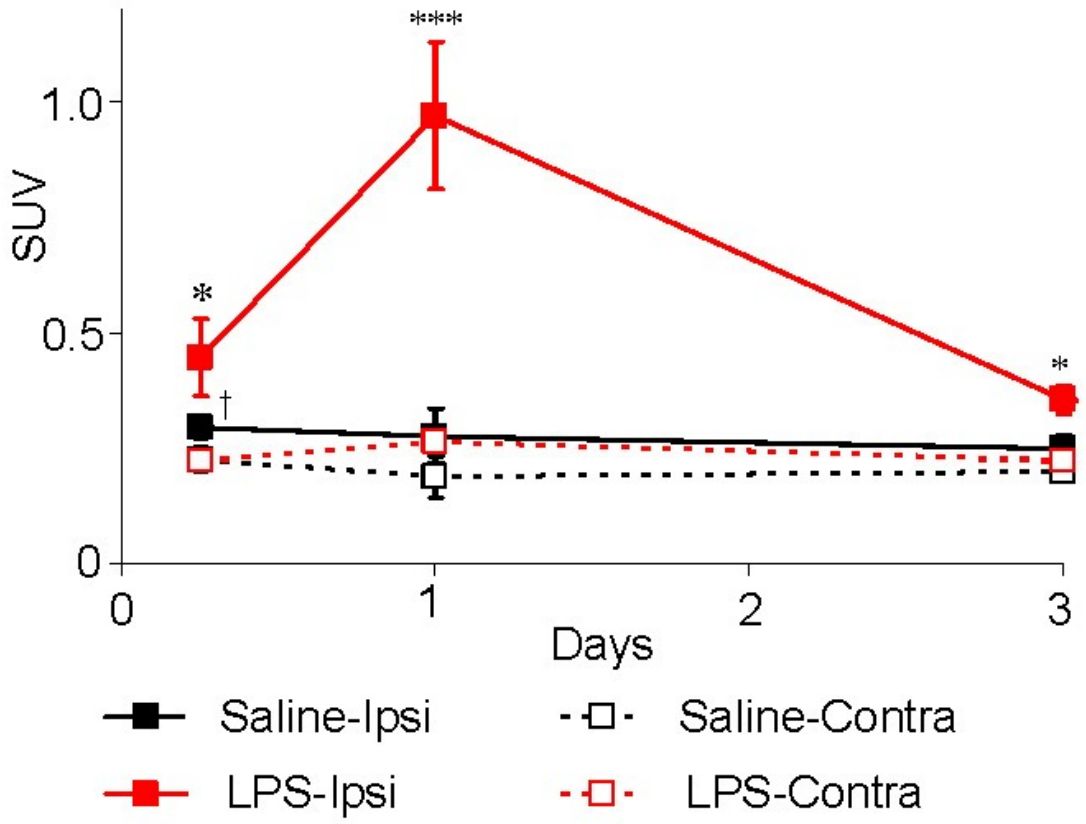
Rats post 1 day after LPS (0.5 μg) injection, and COX-1$^{-/-}$ and COX-2$^{-/-}$ mice were perfused with ice-cold 0.9% saline under deep anesthesia. Brain samples were rapidly frozen in dry-ice powder, and were homogenized in ice-cold lysis buffer (10 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 1 % NP-40, and 1% protease inhibitor cocktail), and then obtained the total protein extract by centrifugation for 10 min at 15,000 rpm at 4°C. Protein concentration was determined by the Bradford assay, and equivalent amount (10 μg for mice and 35 μg for rats) of each sample was subjected to SDS-PAGE electrophoresis. The proteins were transferred onto polyvinylidene difluoride membranes (Hybond-p; GE Healthcare, Amersham) by using Bio-Rad immunoblotting apparatus. The membrane was blocked for 1 h with 20 mM Tris-buffered saline (pH 7.4) containing 2 % Block Ace (Yukijirushi-Nyugyo Co.) and 0.1% Tween 20, and was incubated overnight at room temperature with COX-1 antibody (1:1,000) used in the study of immunohistchemistory or β-actin rabbit polyclonal antibody (Sigma-Aldrich Co., 1:1,000). After several washings, a secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (GE Healthcare, Amersham), was added. Antibody labeling was detected by chemiluminescence and the intensity of the bands was analyzed and normalized as the relative intensity to the band of β-actin.



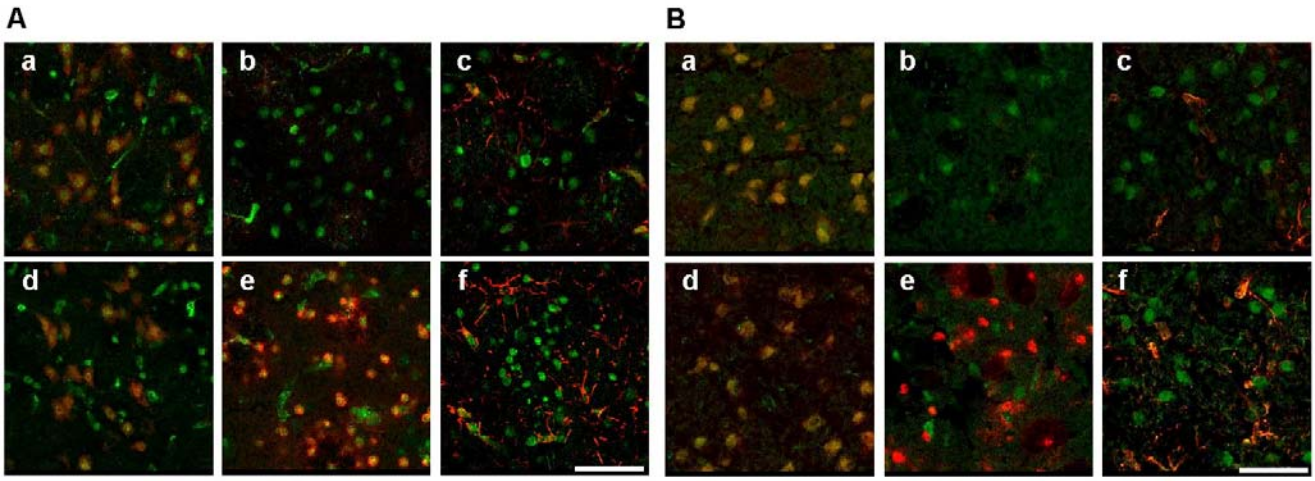
Supplemental Figure 1. Regions of interest (ROI) for quantification of PET images. ROIs were placed on the contralateral (blue) and ipsilateral (red) sides of the striatum according to the MR T1-weighted image. Arrow indicates injection site of LPS or QUIN.



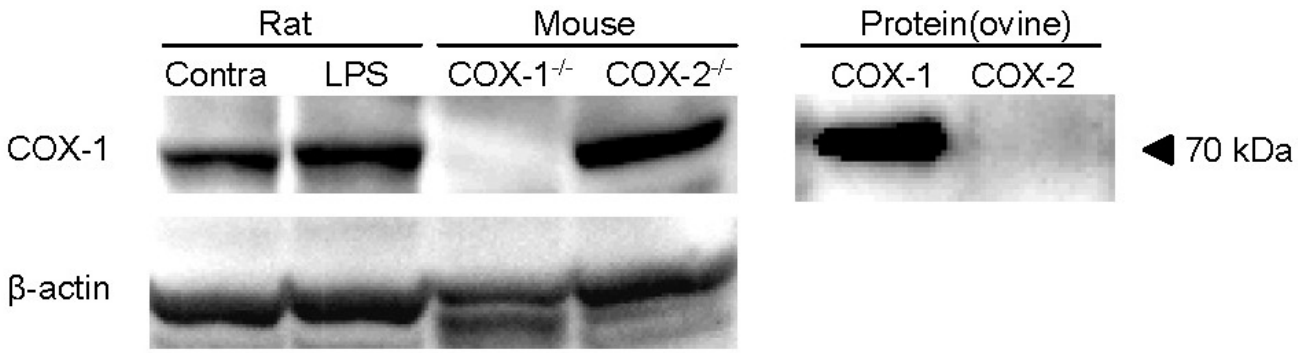
Supplemental Figure 2. Expression of COX-1 and COX-2 in the brain of COX-1$^{-/-}$ and COX-2$^{-/-}$ mice. Representative photomicrographs of COX-1 and COX-2 immunohistochemistry in the hippocampal area of COX-1$^{-/-}$, COX-2$^{-/-}$, and wild-type mice.



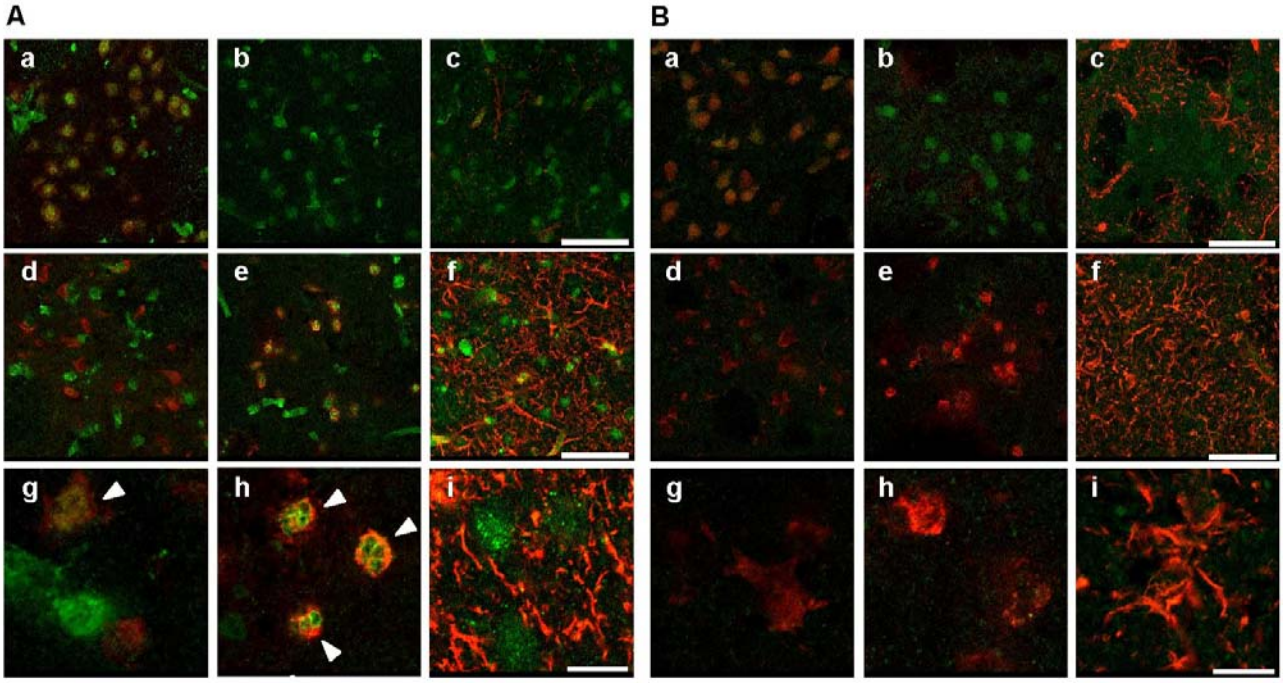
Supplemental Figure 3. Time-radioactivity changes in ^{11}C-KTP-Me accumulation during LPS-induced neuroinflammation, and saline injection. Quantification of radioactivity of ^{11}C-KTP-Me in the rat striatum after LPS (0.5 μg) or saline injection, was performed using summed PET image from 5 to 45 min. Data are expressed as SUV, and the mean ± SD (n = 3 in 6h and 1 day in saline injected rats, n=4 in the others). *p<0.05, ***p<0.001, LPS-ipsilateral striatum *vs.* saline-ipsilateral striatum. †p<0.05, saline-ipsilateral striatum *vs.* saline-contralateral striatum.



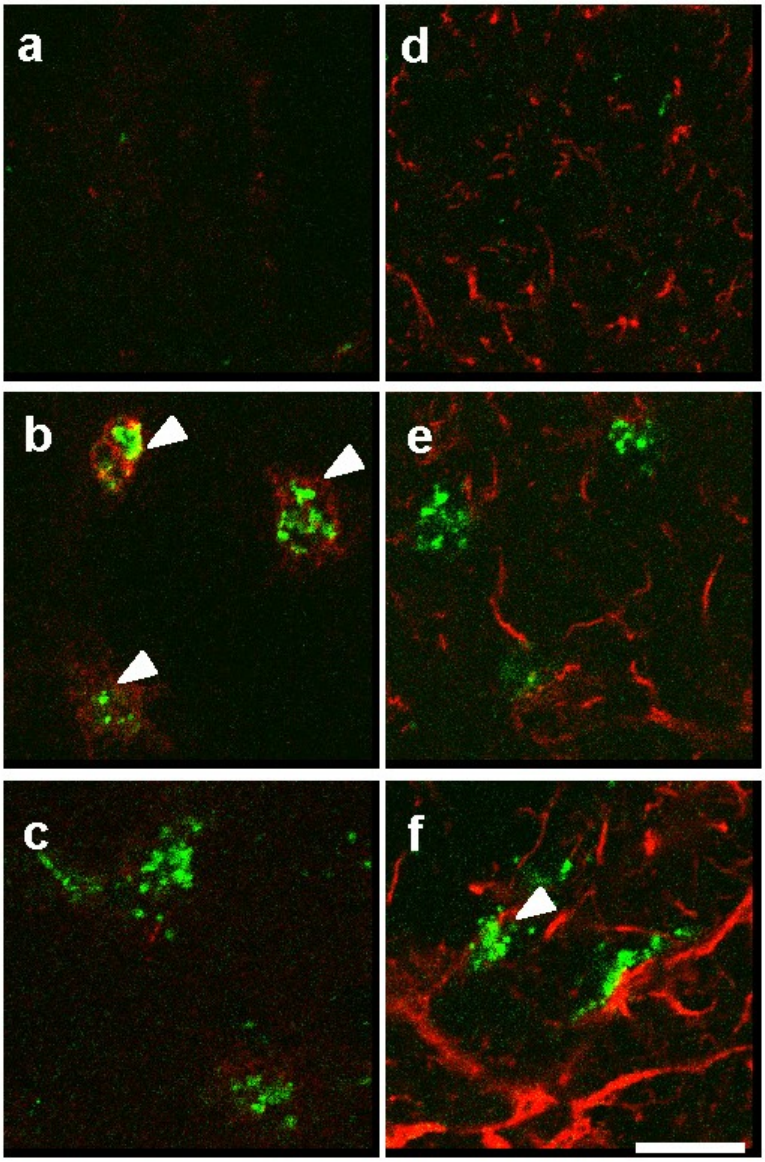
Supplemental Figure 4. Co-expression of COX-1 and COX-2 with the cell type-specific antigens in LPS-induced neuroinflammation (low magnification). Representative photomicrographs of double immunofluorescent labeling of COX-1 (green) and NeuN (a, d; red), and OX-42 (b, e; red) or GFAP (c, f; red) in the striatum at day 1 after LPS (0.5 μg) injection (A). In the contralateral striatum (a-c) only neuronal cells with expression of COX-1 are present (a). In the LPS-injected striatum (d-f) COX-1 is expressed in neuronal cells (d) and in activated microglia/macrophages (e) but not in astrocytes (f). Representative photomicrographs of double immunofluorescent labeling of COX-2 (green) and NeuN (a, d; red), and OX-42 (b, e; red) or GFAP (c, f; red) in the striatum at day 1 after LPS (0.5 μg) injection (B). In the contralateral striatum (a-c) only neuronal cells with expression of COX-2 are present (a). In the LPS-injected striatum (d-f) COX-2 is also expressed in neuronal cells (d) but not in activated microglia/macrophages (e) or astrocytes (f). Scale bar = 50 μm.



Supplemental Figure 5. Representative immunoblot of COX-1 protein in LPS treated rat, and COX-1$^{-/-}$ and COX-2$^{-/-}$ mice. COX-1 protein (70 kDa) was specifically recognized in brain samples of both rat and COX-2$^{-/-}$ mouse, and purified COX-1 protein from ram seminal vesicles, but not in brain sample of COX-1$^{-/-}$ mouse and purified COX-2 protein from sheep placenta. The results strongly demonstrate no cross-reaction of anti-COX-1 antibody with COX-2 protein, and also indicate little increase of COX-1 protein level in LPS injected hemisphere (1.02±0.12 *vs.* contralateral side, n=3). Arrow head indicates the position of marker protein (70kDa).



Supplemental Figure 6. Co-expression of COX-1 and COX-2 with cell-type-specific antigens in the cells during QUIN-induced excitotoxic neurodegeneration. Representative photomicrographs of double immunofluorescent labeling of COX-1 (green) and NeuN (a, d, g; red), and OX-42 (b, e, h; red) or GFAP (c, f, i; red) in the striatum at day 1 after QUIN (60 nmol) injection (A). In the contralateral striatum (a-c) only the neuronal cells express COX-1. In the QUIN-injected striatum (d-i) COX-1 expression is observed in a few neuronal cells (d, arrows in g) and in activated microglia/macrophages (e, arrows in h) but not in astrocytes (f, i). Representative photomicrographs of double immunofluorescent labeling of COX-2 (green) and NeuN (a, d, g; red), and OX-42 (b, e, h; red) or GFAP (c, f, i; red) in the striatum 1 day after QUIN (60 nmol) injection (B). In the contralateral striatum (a-c) only the neuronal cells express COX-2. The expression of COX-2 is decreased in the QUIN-injected striatum (d-i), and co-localization is not detected in the neuronal cells (d, g), activated microglia/macrophages (e, h), or astrocytes (f, i). Scale bars; 50 μm (a-f in the figures A and B), 10 μm (g-i in the figures A and B).



Supplemental Figure 7. The changes in localization of TSPO in glial cells during LPS-induced neuroinflammation. Representative photomicrographs of double immunofluorescent labeling of TSPO (green) and OX-42 (a-c; red) or GFAP (d-f; red) in the striatum after LPS (0.5 μg) injection. In the contralateral striatum (a, d) no expression of TSPO, activated microglia/macrophages or astrocytes are present. At day 1 after LPS injection, TSPO is expressed in activated microglia/macrophages (b) but not in astrocytes (e). At day 7 after LPS injection, TSPO is contrastively expressed in activated astrocytes (f) but not in microglia/macrophages (c). Scale bar = 10 μm.